Alloimmunity Mediated Autoimmunity In Pathogenesis Of Chronic Rejection

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Risk factors associated with BOS

- Alloimmune response
  - Humoral
  - Cellular
- Viral Immune Response
- Primary Graft dysfunction (PGD)
Role for HLA in BOS

- Selected cross match negative transplants
- Retrospective analysis of post transplant sera
- Cytotoxic Antibody development, CDC-AHG
- Correlation with clinical status
Retrospective analysis: Correlation between development of anti-HLA class I antibodies and BOS

Anti-HLA Class I Antibodies precedes the Development of BOS

Anti-HLA Detection

BOS Onset

Time (Months Post-Tx)

P = 0.01
Temporal relationship between increase in ELISA-detected anti-HLA Abs and decrease in pulmonary function after LTx.
Cellular Alloimmune response: Higher Frequency of Donor HLA reactive CD4\(^+\) T Cells in BOS+ Patients

- **BOS+ Class II**: T Cell Frequency (Reciprocal) = 0.000001
- **BOS- Class II**: T Cell Frequency (Reciprocal) = 0.0000002
- **BOS+ Class I**: T Cell Frequency (Reciprocal) = 0.0000008
- **BOS- Class I**: T Cell Frequency (Reciprocal) = 0.0000004

*P = 0.001

*P < 0.05
• Lung transplant patients with BOS develop Anti-donor HLA during the post transplant period

• These antibodies
  – cause complement induced damage.
  – induce proliferation of AEC
  – induce apoptosis of AEC
  – stimulate production of growth factors by AEC
Can anti-HLA induce OAD?

- Animal models
- Tracheal transplantation model
  - Heterotopic
  - Orthotopic
- Limitation… Large Airway Disease
- Development of a new model
Anti-MHC Class I Ab administration into lungs
To drop antibody more accurately, mice were intubated and Abs were administered.

Antibody treatment

BALB/c mice

Anti-H2K^d antibody / C1.18.4 isotype control / anti-keratin ab control

Antibodies administered

Day 0 5 12 19 26

Sacrificed

Fukami et al. , J. Immunology in press
Administration of anti-MHC class I Abs developed significant cellular infiltration around vessel and bronchiole as well as luminal occlusion at 30 days (H&E stain).

A, B, E; Cellular infiltration around bronchiole and vessel
D; Hyperplasia of bronchial epithelium
C; Luminal occlusion
F & G; no lesions
Administration of anti-MHC class I Abs produced significant fibroproliferation at 15 and 30 days (Trichrome stain)

H2K\textsuperscript{d}

C1.18.4 isotype control

A,C,D; Fibroproliferation around bronchiole
B; Occlusion lesion

E,F; No fibrosis
No lesions following administration of anti-keratin antibody

15 day

30 day

H&E

Trichrome
• Anti-MHC administration directly into the lung can result in
  – Epithelial metaplasia
  – Cellular infiltration
  – Endothelitis
  – Luminal occlusion
• Increases Cytokines and chemokines in the lung tissue as well as several growth factors.
Can anti-MHC antibody induce autoimmunity?

Cellular: ELISPOT

K-α 1 tubulin and collagen V

Humoral: ELISA

K-α 1 tubulin and collagen V
Increased cytokine production against self antigens by lymphocytes from lungs of mice treated with anti-MHC antibody.
Administration of Anti-MHC class I Abs leads to development of autoantibodies
Increased IL17 production against self antigens by lymphocytes from lungs of mice treated with anti-MHC antibody.
Effect of neutralization of IL-17

- Administer anti-H2kd endobronchially on day 1, 2, 3, 5 and weekly thereafter
- Administer 100 μg of anti-IL-17 antibody intravenously on day 0, 3, 6 and 9
- Day 30
  - Sera for quantitation of autoantibodies
  - Lungs for histopathological analysis
Neutralization of IL-17 decreases cellular infiltration, epithelial metaplasia and fibrosis

Anti-MHC

Anti-MHC + anti-IL17
ROLE OF B CELLS and ANTIBODIES in the INDUCTION OF AUTOIMMUNITY and OAD
Administration anti-MHC class I Abs to B^-/- mice showed a significant decrease in the frequency of IFN-g secreting T cells.
Administration anti-MHC class I Abs to B^{-/-} mice showed a significant decrease in the frequency of IL17 secreting T cells.
Administration of anti-MHC class I Abs to B−/− mice had a reduced cellular infiltration bronchiole epithelium damage compared to Isotype at 30 days.

Isotype Abs

B−/− Mice

H2Kb

Iso type showed a normal bronchiole epithelium and no cellular infiltration. WT showed a significant cellular infiltration in peribronchiole and perivascular and luminal occlusion. Closed arrow is luminal occlusion. Open arrow is cellular infiltration.

Original magnification is X200 B; Bronchiole  V; vessel
Administration of anti-MHC class I Abs to B^-/- mice didn't induce development of fibrosis in peribronchiole and perivascular compared to WT at 30 days.

Closed arrow is luminal occlusion.
Open arrow is fibrosis
Original magnification is X200 B; Bronchiole V; vessel
Summary

Anti-MHC Class I Ab can:

- Lead to the exposure of cryptic antigens or their determinants
- Lead to immune responses to self antigens, IFN gamma and IL-17 production
- Lead to the development of antibodies to Kα-1tubulin and collagen V
  - Upregulate Chemokines & their Receptors
  - Up-regulate Growth factors
  - Antibodies to self antigens play an important role in the pathogenesis.
- B-CELLS ARE IMPORTANT ANTIGEN PRESENING CELL.
Development of Abs to K- α 1 tubulin and collagen V in LTx recipients developing anti-HLA allo-Abs.

<table>
<thead>
<tr>
<th>Patients</th>
<th>k α 1 tubulin Abs (μg/ml)</th>
<th>Collagen V Abs (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=10)</td>
<td>132±43</td>
<td>92.6 ± 34.2</td>
</tr>
<tr>
<td>HLA- (n=10)</td>
<td>147.5 ± 94.5</td>
<td>190.3 ± 113.9</td>
</tr>
<tr>
<td>HLA+ (n=10)</td>
<td>458.9 ± 342.7</td>
<td>365.7 ± 219.8</td>
</tr>
</tbody>
</table>
Persistence of Abs to self antigen in the absence of anti-DSA.
Proliferation of BOS+ PBLs against self antigens K-α1 tubulin or Collagen V.

- **Unstimulated**
- **K α 1 tubulin**
- **Collagen V**
BOS+ Ltx patients show decreased IL-10 and higher IFNγ, IL-17 production against K-α1 tubulin.
Summary

• A predominance of IL-10 producing T cells reactive to col-V with lower levels of IFN-γ and IL-2 producing T cells.

• The col-V specific T(IL-10) cells suppressed the proliferation of col-V specific cells by IL-10-dependent and contact-independent pathways.

• During chronic rejection there is a significant decline of IL-10 producing T cells with concomitant expansion of K α-1 tubulin and col-V-specific IFN-γ producing cells.

• BOS + HLA+ serum also had auto antibodies to K α-1 tubulin and Col-V.
Evidence for auto-immunity to self antigens in chronic rejection following heart and kidney transplantation.

Development of Immune responses:
- Myosin following heart transplantation
- Vimentin following heart and kidney transplantation
- Collagen IV following kidney transplantation
- Collagen V and K alpha tubulin in BM Transplant recipients with chronic GVHD and Pulmonary insufficiency.
Development of Abs to Collagen IV in post- Kidney Transplant sera

**Graph 1:**
- **X-Axis:** No changes (N=5) vs. Chronic changes (N=12)
- **Y-Axis:** Concentration of Col IV Abs (ng/ml)
- **Legend:**
  - Squares: No changes
  - Triangles: Chronic changes
- **Statistical Information:** P<0.05

**Graph 2:**
- **X-Axis:** Normal biopsy (N=5) vs. Chronic changes (N=12)
- **Y-Axis:** Concentration of Col IV AutoAbs (ng/ml)
- **Legend:** Normal biopsy
- **Statistical Information:** P<0.05
Development of Abs to Vimentin in post kidney Transplant Sera

- P<0.05

Graph 1:
- Vimentin AutoAbs Conc. μg/ml
- No Changes N=5
- Chronic Changes N=12

Graph 2:
- Vimentin AutoAbs Conc. mg/ml
- Normal biopsy N=5
- Chronic Changes N=12

P<0.05
## Correlation between development of DSA and Abs to Col IV and Vimentin in Renal Tx Recipients

<table>
<thead>
<tr>
<th>Renal Transplant recipients</th>
<th>HLA/DSA Antibodies</th>
<th>Coll IV autoAbs</th>
<th>Vimentin AutoAbs</th>
<th>C4d staining</th>
<th>Chronic Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Human Serum (15)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R1</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>present</td>
</tr>
<tr>
<td>R2</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>R3</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>R4</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Present</td>
</tr>
<tr>
<td>R5</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>present</td>
</tr>
<tr>
<td>R6</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>R7</td>
<td>DSA+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>R8</td>
<td>HLA+ (Class II)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Present</td>
</tr>
</tbody>
</table>
Summary

- Recipients with Chronic changes in renal biopsy were often positive for Abs to Col IV and Vimentin.
- DSA + patients 7/12 were positive for Abs to self antigens.
- Few clinically stable recipients were positive for Abs to Col IV and vimentin as well as anti-HLA.
- Sera from healthy volunteers do not have Abs to HLA or self antigens.
Conclusion

- Allo-immune responses can lead to immune responses to self antigens.
- There is a loss of peripheral tolerance.
- Auto-immune responses alone or in conjunction with allo-immune responses can lead to chronic rejection
Antibodies to MHC, Allo-immune responses, Viral immune responses:

**Induces autoimmunity**

Play a significant role in the pathogenesis of chronic rejection
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